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Association in Vivo of Glycated Apolipoprotein A-I with High Density Lipoproteins

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Summary: In diabetic patients, hyperglycaemia results in the non-enzymatic glycation of apolipoprotein A-I, the major protein of human high density lipoproteins.

The effect of the non-enzymatic glycation on the association of apolipoprotein A-I with high density lipoprotein in vivo has been studied in the rat.

The distribution volume obtained after injection of glycated apolipoprotein A-I was 2- to 3-fold higher in kidneys and approximately 30% lower in adrenals and ovaries than that obtained with apolipoprotein A-I.

Analysis by gel chromatography of serum from donor rats shows that glycation diminishes the interaction between apolipoprotein A-I and high density lipoprotein.

The findings in this study suggest that non-enzymatic glycation of apolipoprotein A-I may contribute to the development of atherosclerosis in patients with diabetes mellitus.

Introduction

Excessive non-enzymatic glycation of protein has been implicated in the development of several complications of diabetes mellitus, including atherosclerotic vascular disease (1).

Lipoprotein abnormalities are related to the development of atherosclerosis and, therefore, non-enzymatic glycation of the protein moiety of lipoproteins (apolipoproteins) has been the subject of intensive study.

Increased non-enzymatic glycation of all major classes of apolipoproteins has been demonstrated in the plasma of diabetic patients (2, 3).

Alterations of the self-association and lipid-binding properties of apolipoprotein A-I, resulting from its non-enzymatic glycation, have been reported in vitro (4), suggesting that this modification in the apolipoprotein A-I may affect the structural cohesion of high density lipoprotein (HDL) particles.

In this report we document an abnormal association of glycated apolipoprotein A-I with high density lipoprotein in vivo in the rat.

Materials and Methods

Isolation of high density lipoprotein, apolipoprotein A-I and glycated apolipoprotein A-I

Human high density lipoprotein (d = 1.063 – 1.21 kg/l) was isolated by sequential ultracentrifugation (5). Apolipoprotein A-I was purified from high density lipoprotein using well established procedures (6). Glycated apolipoprotein A-I was purified from the plasma of diabetic patients as previously described (4).

Binding in vivo of glycated [^{125}I]apolipoprotein A-I and [^{125}I]apolipoprotein A-I to organs

Glycated apolipoprotein A-I (1 – 2 glucose molecules per apolipoprotein molecule) and apolipoprotein A-I were radioiodinated with ^{125}I by the chloramine T method (7). For experiments involving reassociation of apolipoprotein A-I with high density lipoprotein in vivo, each labelled apolipoprotein was injected

via the femoral vein into adult female rats. Two hours later, the animals were exsanguinated. Serum was immediately separated and reinjected into acceptor rats for tissue binding studies. The acceptor rats were exsanguinated via the abdominal aorta 5–7 min after injection. The liver, kidneys, adrenals, ovaries, spleen, abdominal muscle, heart, lungs and stomach were removed (normally within 10 min of the injection).

The organs were cleaned, weighed and counted for ^{125}I. The results were expressed in terms of specific tissue distribution volumes (DV):

$$DV\ (\mu l/g\ tissue) = \frac{counts/min\ per\ g\ organ}{counts/min\ per\ \mu l\ of\ serum} \quad (8)$$

The non-specific association of radioactivity with the organs, due to the presence of residual serum after removal of organs, was estimated by measurement of organ distribution volumes of albumin in a group of control rats.

Study of association of ^{125}I-labelled apolipoproteins with high density lipoprotein

The association of glycated apolipoprotein A-I or apolipoprotein A-I with high density lipoprotein in vivo was determined by gel filtration chromatography. The serum obtained two hours after injection of either glycated [^{125}I]apolipoprotein A-I or [^{125}I]apolipoprotein A-I in a donor animal was applied to a column (0.9 × 50 cm) of Sephadex G-150, and eluted with 0.05 mol/l barbital buffer, pH 8.6. Fractions of 0.5 ml were collected for measurement of radioactivity.

Statistical analysis

Differences between means were tested with *Student*'s t test.

Results

The specific tissue distribution volumes obtained after injection of radioiodinated glycated apolipoprotein A-I or apolipoprotein A-I are shown in figure 1.

Only four tissues (liver, kidneys, adrenals and ovaries) were specifically labelled and little or no binding was detected in the remaining organs.

The adrenals and ovaries were the predominant organs of glycated apolipoprotein A-I and apolipoprotein A-I binding. However, the binding of glycated apolipoprotein A-I was significantly less ($p < 0.01$) than the binding of apolipoprotein A-I in these organs.

There was no difference between the distribution volumes of both apolipoproteins in liver.

Of particular interest, the distribution volume determined with glycated apolipoprotein A-I in the kidneys was 2- to 3-fold higher than the determined with apolipoprotein A-I. Previous studies (8, 9) have proposed that the uptake of apolipoprotein A-I by kidneys may involve free, rather than high density lipoprotein-bound apolipoprotein A-I.

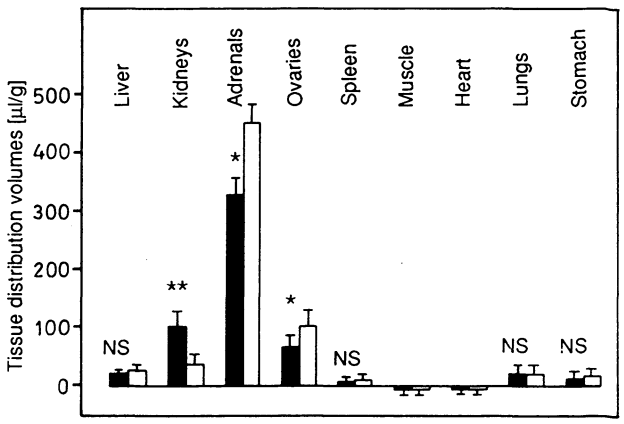

Fig. 1. In vivo binding of glycated apolipoprotein A-I (■) and apolipoprotein A-I (□) to organs in the rats. The tissue distribution volumes were determined after injection of the radioiodinated apolipoproteins as described under Materials and Methods. The results expressed in µl/g of tissue are corrected for the tissue distribution volume of radioiodinated albumin, considered as representative of the non-specific association. Each value is the mean ± SEM for 10 animals (**, $p < 0.001$; *, $p < 0.01$; NS, not significant).

Gel filtration of serum obtained from a donor rat injected with either glycated [^{125}I]apolipoprotein A-I or [^{125}I]apolipoprotein A-I for reassociation with high density lipoprotein in vivo shows that virtually all of the apolipoprotein A-I was associated with high density lipoprotein; and very little free apolipoprotein A-I was detected. However, when glycated apolipoprotein A-I was reassociated with high density lipoprotein, only 70% of radioactivity was associated with this lipoprotein while the rest remained as free apolipoprotein A-I (fig. 2).

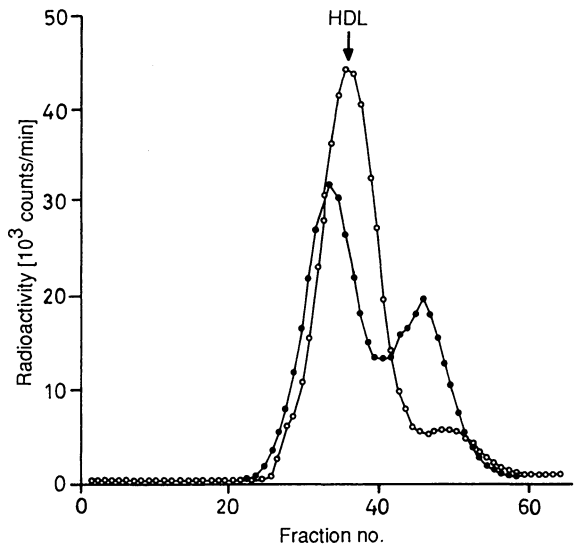

Fig. 2. Sephadex G-150 gel filtration chromatography of serum obtained 2 h after injection of glycated [^{125}I]apolipoprotein A-I (●) or [^{125}I]apolipoprotein A-I (○) into a donor rat. The arrow denotes the elution volumes of HDL determined in independent calibration runs.

Discussion

This study shows that glycated apolipoprotein A-I from diabetic patients behaves differently from the non-glycated form when injected into rats. The high uptake of apolipoprotein A-I by adrenals and ovaries is consistent with previous reports that high density lipoproteins are the main suppliers of cholesterol for steroidogenic tissues in the rat (10, 11).

The smaller uptake of glycated apolipoprotein A-I compared with apolipoprotein A-I in these organs may reflect the diminished association of glycated apolipoprotein A-I with high density lipoprotein; our results agree with those of other authors, who established that adrenals and ovaries bind high density lipoprotein-bound apolipoprotein A-I (8). In contrast, the preferential uptake of glycated apolipoprotein A-I by kidneys cannot be explained by uptake of intact high density lipoprotein particles, but may rather be attributed to glomerular filtration and tubular reabsorption of apolipoprotein A-I that is not associated with high density lipoprotein (9). This is consistent with the large amount of free glycated apolipoprotein A-I detected chromatographically in plasma of donor rats injected with this apolipoprotein, and it suggests that free and lipoprotein-bound apolipoproteins may have different catabolic sites. It was not the purpose of this study to establish the nature of this binding. The most reasonable explanation is that in these tissues the high density lipoprotein binding is mediated by apolipoprotein A-I (12).

The mechanism by which the non-enzymatic glycation may alter the association of apolipoprotein A-I with high density lipoprotein is not clear; it is possible, however, that the presence of covalently-linked glucose molecules on lysine residues in an amphipathic domain of apolipoprotein A-I could alter the lipid-protein interaction.

Thus, this abnormality in glycated apolipoprotein A-I might contribute to the development of atherosclerosis in patients with diabetes mellitus.

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